An Analysis of the Mechanical Responses of the Isolated Ileum to Single Transmural Stimulation and to Drugs

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INTRODUCTION

In the study on the innervation of intestinal smooth muscle, transmural stimulation of the intrinsic nerves has been commonly employed. Intramrual, repetitive stimulation to the intrinsic inhibitory nerves produced various patterns of the mechanical responses of the intestinal segments[1),2)]. The membrane potential of smooth muscle cells was increased by intramural stimulation of the intrinsic inhibitory nerves and the hyperpolarization was again recovered during the repetitive stimulation[2)-7)]. Therefore, the mechanical response produced by the repetitive stimulation to the intramural inhibitory nerves was due not only to the activity of intrinsic nerves but also to rebound excitation. In fact, the considerable contraction of the guinea-pig colon occurred even though stimulation to intrinsic inhibitory nerves continued[2)]. According to the preceding view, single transmural stimuli to the intrinsic fibers are more useful to study the innervation of the intestinal smooth muscle when the changes in the mechanical response are employed as the characteristics of nerves.

In the guinea-pig colon, it has been generally accepted that three kinds of intrinsic fibers exist in the intestinal wall. It has also been shown recently that adenosine triphosphate (ATP) or its related nucleotides may be the transmitter substance released by the non-adrenergic inhibitory nerves in the gut[8)-11)]. However, the effects of ATP on the guinea-pig ileum was complex. In contrast with the inhibitory responses of most preparations of mammalian gut, the excitatory or diphasic effect of ATP has been reported[9),10)].

In the present experiment, the innervation of the guinea-pig ileum was examined by single transmural stimulation to intrinsic nerves. The rebound-contraction in the guinea-pig ileum was also examined. The mechanical responses to single transmural stimuli were used to estimate the function

of the intrinsic nerve fibers.

METHODS

Guinea-pigs of either sex, weighing between 200 and 500g, were stunned and bled out. The small intestine was dissected. Sections of ileum were suspended in a 300 ml of organ bath and connected to an electronic transducer. The tension of the preparation was between 0.5 and 2g. The preparation was bathed in the solution described in the previous paper[12] at 36°C and bubbled with 95% O_2 and 5% CO_2. Tension recording systems were the same as those described in the previous paper[12]. To stimulate the intestinal wall, a silver wire was inserted into the lumen of segment and a reference electrode was placed around the segment of small intestine. Rectangular pulses were delivered from an electronic stimulator (MSE-40, Nihon Kohden). The pulse duration was usually 0.1–0.01 msec. The drugs used, freshly dissolved in the modified Krebs solution, were; adenosine-5′-diphosphate trisodium (ADP), adenosine-5′-triphosphate disodium (ATP), atropine sulfate, d-tubocurarine chloride, guanethidine sulfate, hexamethonium bromide, hyoscine hydrochloride, phenoxybenzamine hydrochloride, physostigmine sulfate, procaine hydrochloride and tetrodotoxin.

RESULTS

After long exposure to the normal solution, the guinea-pig ileum showed spontaneous contractile activity but the activity was irregular in magnitude as well as in the interval between the successive contractions. At the initial stage of the experiment, the preparation was not active mechanically. However the resting tension was not quiescent and the minor fluctuation in the resting tension was observed. At this time, when a single transmural stimulation was given to stimulate the intrinsic nerves, a mechanical response could be produced in all preparations. When the interval between single transmural stimulations was longer than 5 sec, the constant responses were obtained during long period repeatablely.

Single transmural stimulation in normal solution

When single transmural stimuli of the pulse duration less than 0.1 msec, were applied, a response of the segment of guinea-pig ileum was usually obtained and its magnitude of the tension was relatively constant. The response consisted of mixed excitatory and/or inhibitory components. Responses are classified into two types.

First type of the response was a contraction type, that is, contractile response was not preceded by inhibition. This type might correspond to

a primary contraction defined by Furness[2] in the guinea-pig colon. The contraction of this type was conventionally termed "primary contraction" in the present experiment. The delay between the single pulse and the start of the contraction was less than 0.2 sec. The magnitudes of the primary contraction were nearly equal as shown in Fig. 1A. The contraction was sometimes follwed by a period of tension decrease.

Second type of response was a relaxation type, that is, the relaxation produced initially. In general, a slow contraction followed after the initial relaxation. Furness[2] had defined as secondary contracion which occurred on cessation of repetitive stimulation. This slow contraction might be correspond to a rebound excitation. In the present experiment, the contraction of this type was termed "rebound-contraction". The delay between the single pulse and the start of the rebound-contraction was usually less than 2 sec. The time course and the magnitude of each initial relaxation in one segment produced by transmural stimulations at long intervals (longer than 5 sec) were similar, as shown in Fig. 1B. The rate of rise of rebound-contraction was lower than that of the primary contraction, of the phasic contraction produced by an external electrical stimulation

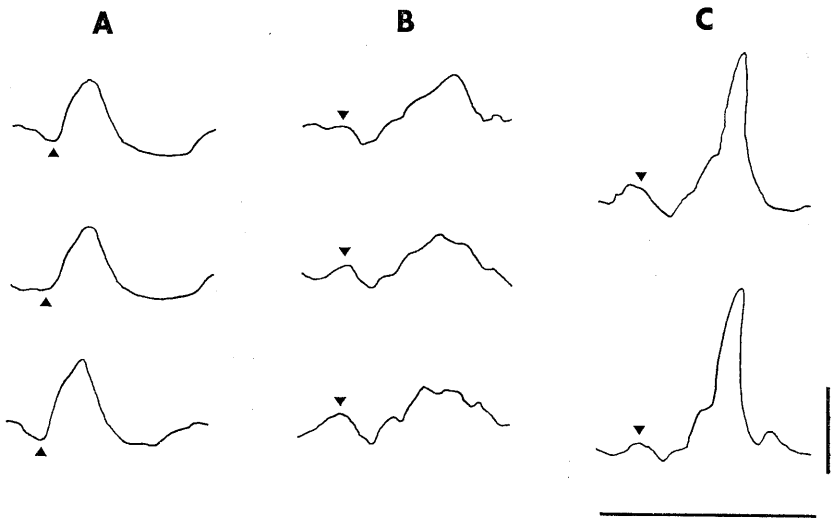

Fig. 1. Responses of isolated segments from the guinea-pig ileum to single transmural stimulation of intrinsic nerve fibers in normal solution. A; Contraction type response, the primary contraction was sometimes followed by a period of lowered activity. B; Relaxation type response, the relaxation was produced initially. The rebound-contraction followed the initial relaxation. C; Relaxation type response with a phasic, spontaneous contraction on the rebound-contraction. The duration of single pulse was 0.1 msec in each panel. The pulses were given at the marks (black triangle). Calibration, 10 sec and 0.2 g.

and of the spontaneous phasic contraction. The maximum tension of the rebound-contraction was usually smaller than that of the primary contraction. The magnitude and the time course of the rebound-contraction in relaxation type-preparation were similar. When the duration of the transmural stimuli was varied from 0.01 msec to 0.1 msec, the magnitude of the rebound-contractions was increased with the duration of stimulation. In some preparations, a phasic contraction was spontaneously generated on the rebound-contraction as shown in Fig. 1C. The time course of this phasic contraction was different from that of the rebound-contraction. These types of responses were shown in Fig. 1.

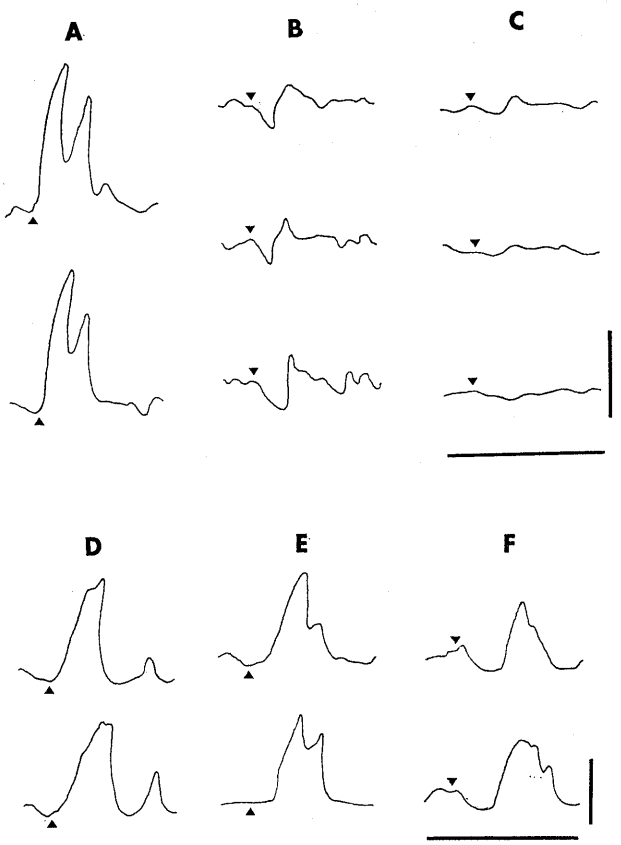

Fig. 2 Effects of atropine and d-tubocurarine on the responses produced by single transmural stimulation. A; Control. The contraction type responses were seen in normal solution. B; After atropine 10^{-6}g/ml, the initial relaxations and the rebound-contractions were produced. C; TTX (1.6×10^{-7}g/ml) abolished both initial relaxation and rebound-contractions in the presence of atropine. D; Control. E; The response type was not altered in d-tubocurarine 10^{-6}g/ml. F; After d-tubocurarine, the addition of atropine (10^{-6}g/ml) produced the relaxation type response to single, equal transmural stimulation. The single pulse, 0.05 msec duration, was given at the mark. Calibration, 10 sec and 0.5 g in each panel.

When the duration of single transmural stimulation ranged from 0.01 msec to 3 msec in one prepararation, different responses were obtained. Weak stimulations (0.01–1 msec) produced the relaxation type responses. Large contractions were produced by strong stimulations (2–3 msec) without the initial relaxation and the spontaneous phasic contractions were generated after the large contraction. This large contraction was different from the primary contraction in time course and magnitude of tension development. This response might be produced by a direct effect on intestinal smooth muscle.

Atropine and hyoscine

Atropine in concentration of 10^{-9}g/ml inhibited the spontaneous

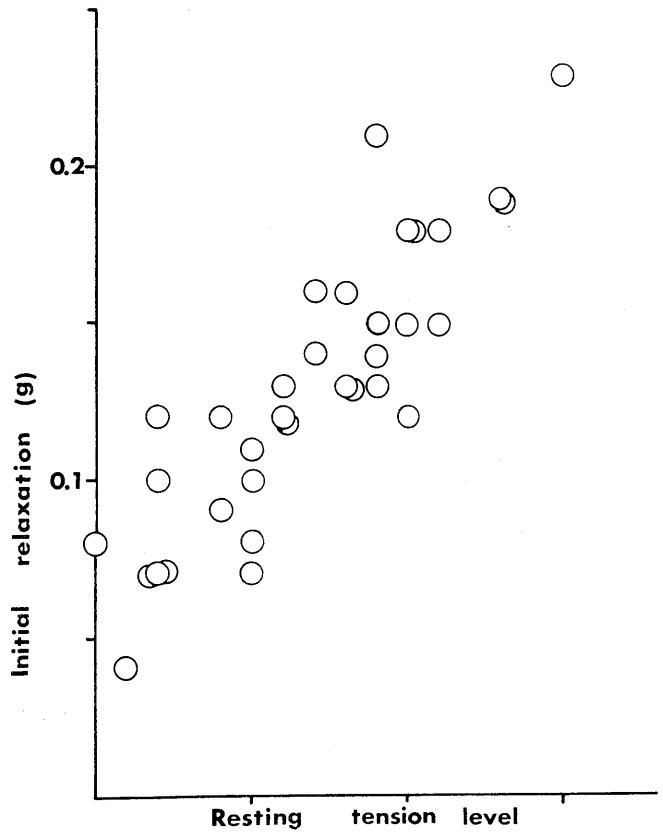

Fig. 3. Relationship between the resting tension level at which the single transmural stimulation to intrinsic inhibitory nerves was given and the the magnitude of the initial relaxation. The experiment was made in the presence of hyoscine (10^{-5}g/ml). The stimulations (0.1 msec in duration) were applied at the long interval (over than 10 sec) but randomly. The resting tension level is in arbitrary unit.

activity initially. The primary contraction observed in normal solution was also blocked by atropine ($10^{-6}-2\times10^{-6}$g/ml). After the block of the primary contraction, the relaxation type of the response was produced (Fig. 2). The initial relaxation was produced from various tension level because the resting tension fluctuated in atropine. The maximum relaxation levels of each response were nearly equal. The magnitude of the response was depended on the tension level at which the single transmural stimulation was given. The relationship between the resting tension level and the magnitude of the initial relaxation was shown in Fig. 3.

In atropine (10^{-6}g/ml), single transmural stimulation produced the relaxation type response in all preparations. The initial relaxation may be produced by the action of intrinsic inhibitory nerves. In many preparations, the initial relaxation was followed by the rebound-contraction. The

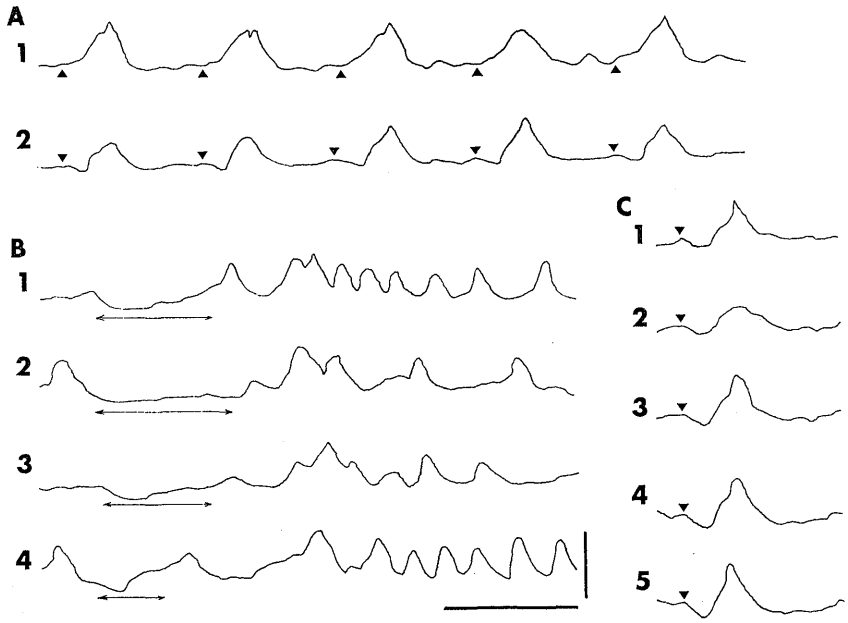

Fig. 4. Effect of hyoscine (10^{-5}g/ml) on the responses to single and repetitive stimulation to intrinsic inhibitory nerves. A 1; Control, the primary contractions were produced by single stimulation. A 2; After hyoscine (10^{-5}g/ml), the initial relaxations were produced. B; Repetitive stimulation (1, 1/sec; 2, 2/sec: 3, 4/sec and 4, 10/sec) produced the continuous relaxation in hyoscine (10^{-5}g/ml). C; Effects of the increase in the strength of single stimulation (1, 10V; 2, 20V; 3, 30V; 4, 40V and 5, 50V) on the initial relaxation and the rebound-contraction in hyoscine (10^{-5}g/ml). The duration of single stimulation was 0.05-0.1 msec. The single pulse was given at the mark (A and C). The repetitive stimulations were applied during the underbars in B. Calibration, 10 sec and 0.5g.

magnitudes of the rebound-contraction were nearly equal in one segment.

Repetitive stimulation (1/sec–10/sec) to the intrinsic inhibitory nerves produced continuous relaxation. In many cases, the tension gradually increased during the stimulation. The rebound-contraction was generated after the cessation of the repetitive stimulation. The after-contraction was followed by the high spontaneous activity.

Hyoscine is known to have an inhibitory effect on an excitatory junction potential in intestinal smooth muscle[3]. Hyoscine (5×10^{-6}–10^{-5}g/ml) also inhibited the primary contraction and the relaxation type response was produced after 2–3 min (Fig. 4). If the relaxation type response was observed in normal solution, the response was not blocked by hyoscine. With increasing strength of the single transmural stimuli, the magnitude of the initial relaxation was also increased (Fig. 4). The rebound-contraction was also accompanied by the initial relaxation in hyoscine. The initial relaxation in hyoscine may due to stimulate to the intrinsic inhibitory nerves. Repetitive stimulations (0.1 msec in duration and 1/sec, 2/sec, 4/sec and 10/sec) produced the continuous relaxation but the tension was slightly and gradually increased during repetitive stimulation. When the repetitive stimulation ceased, the after-contractions occurred in each case (Fig. 4). After atropine (10^{-6} g/ml), the addition of hyoscine (10^{-5}g/ml) potentiated the initial relaxation.

When d-tubocurarine (10^{-6}g/ml) was applied, the primary contraction was not blocked. However, after d-tubocurarine, the relaxation type response was observed after the additional application of atropine (10^{-6}g/ml). The magnitude of the primary contraction was potentiated by physostigmine (10^{-6}g/ml). If the relaxation type response was seen in normal solution, the magnitude of the initial relaxation was decreased and the rebound-contraction was potentiated in physostigmine (10^{-6}g/ml). The rate of rise in the tension development of the rebound-contraction was also increased.

Tetrodotoxin and hexamethonium

Tetrodotoxin (1.6×10^{-6}g/ml) showed an inhibitory effect on the spontaneous contractile activity of the guinea-pig ileum as reported previously[17]. Both contraction and relaxation type responses in normal solution were blocked by tetrodotoxin (0.8–1.6×10^{-6}g/ml). After atropine (10^{-6}g/ml), the initial relaxation and the rebound-contraction were inhibited gradually by tetrodotoxin (0.8–1.6×10^{-6}g/ml), both were finally abolished. Hexamethonium (10^{-5}–10^{-4}g/ml) had no effect on the primary contraction and the relaxation type response in normal solution. After hexamethonium (10^{-4}g/ml), the addtional application of tetrodotoxin (0.8×10^{-6}g/ml) blocked the initial relaxation and the rebound-contraction. Fig. 5 shows the effects of

TTX and hexamethonium on the response to single transmural stimulation.

Guanethidine and phenoxybenzamine

For the contraction type of preparation, guanethidine (10^{-5}g/ml) had no effect on the primary contraction. The initial relaxation observed in normal solution or after atropine (10^{-6}g/ml) was not blocked by guanethidine (10^{-5}g/ml). The rebound-contraction was also observed in guanethidine. In some preparations, the rebound-contraction was slightly potentiated by the addition of guanethidine (10^{-5}g/ml). Phenoxybenzamine (10^{-6}g/ml) had an inhibitory effect on the initial relaxation observed in normal solution or after atropine (10^{-6}g/ml). When the initial relaxation was inhibited, but not abolished completely by phenoxybenzamine, the magnitude of the rebound-contraction was also decreased. The effect of phenoxybenzamine is shown in Fig. 6.

A blocking effect of procaine on the inhibitory junction potential of the intestinal smooth muscle cell membrane has been reported[6),7)]. Procaine

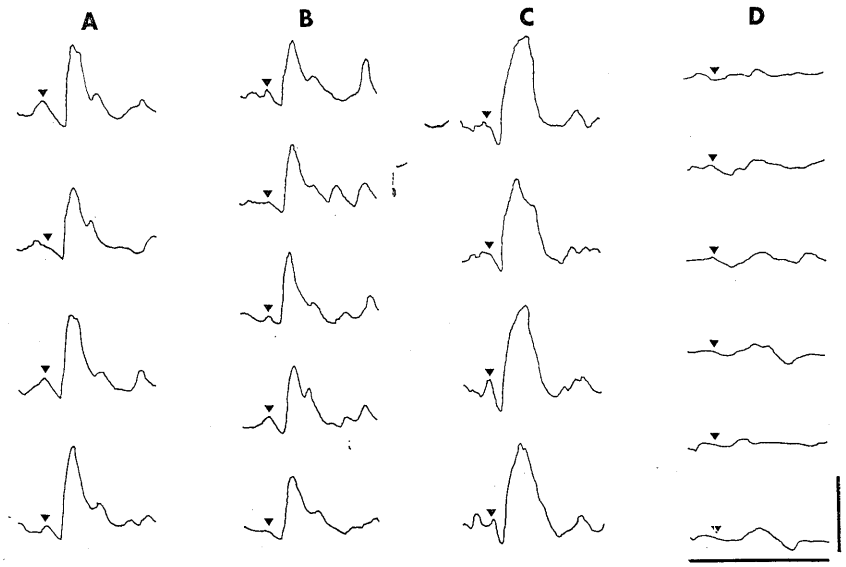

Fig. 5. Effects of hexamethonium and TTX on the response produced by single transmural stimulation. A; Control, the relaxation type responses were seen in normal solution. B; Hexamethonium (10^{-5}g/ml). C; Hexamethonium (10^{-4}g/ml). The initial relaxation and the rebound-contraction in atropine were not abolished by hexamethonium. D; TTX (0.8×10^{-6}g/ml) added after hexamethodinium (10^{-4}g/ml). The initial relaxation and the rebound-contraction were inhibited and finally abolished in TTX. The duration of the single pulse given at the mark was 0.1 msec. Calibration, 10 sec and 0.5g.

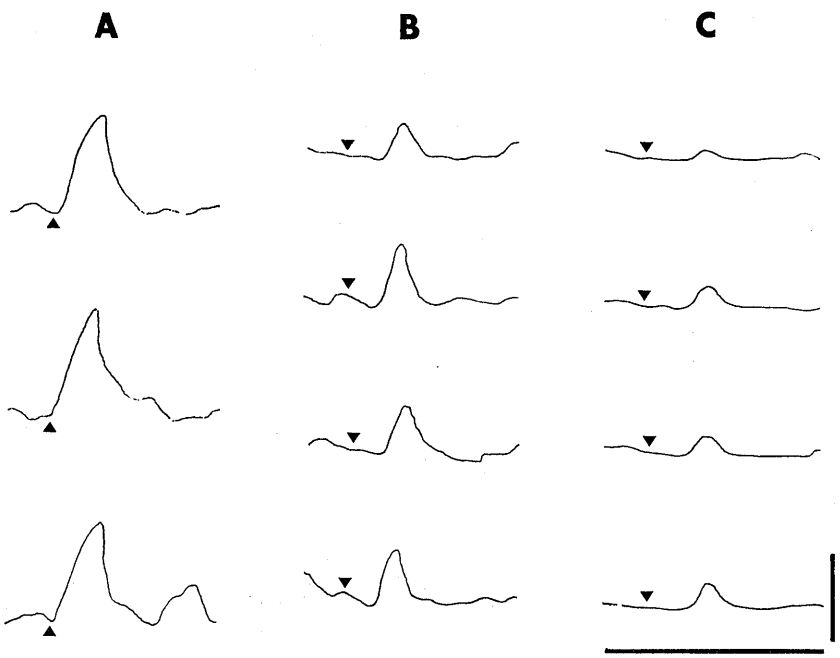

Fig. 6. Effect of phenoxybenzamine on the response produced by single transmural stimulation.
A; Control. The contraction type response was seen in normal solution. B; After atropine (10^{-6}g/ml), the relaxation type response was produced by single pulse. C; Additional application of phenoxybenzamine (10^{-6}g/ml) caused the inhibition on the initial relaxation and the rebound-contraction. The single pulse, 0.05 msec in duration, was given at the mark. Calibration, 10 sec and 0.5g.

was tested on the initial relaxation. Procaine in concentrations of 10^{-5}–3×10^{-5}g/ml failed to block the initial relaxation and the rebound-contraction. However the rate of rise of the rebound-contraction was increased and the spontaneous phasic contraction on the rebound-contraction was seen. The spontaneous activity was increased in procaine (10^{-5}–3×10^{-5}g/ml). In high concentration of procaine (10^{-4} g/ml), the contracture was produced initially and then the tension gradually recovered to the original level but it took a long period. Single transmural stimulation could not trigger the response at this time.

ATP and ADP

Recently the importance of purinergic fibers in the gastrointestinal tract was emphasized by many authors[8),9),10)]. After atropine (10^{-6}g/ml), the additional application of ATP (10^{-5}g/ml) potentiated the initial relaxat-

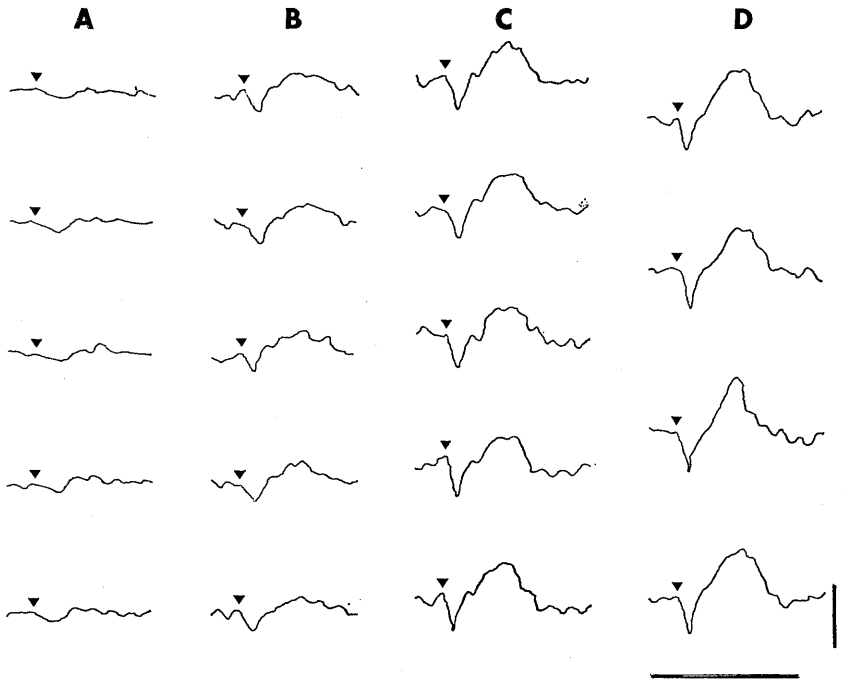

Fig. 7 Effect of ADP on the initial relaxation. In normal solution, the contraction type response was observed. A; The initial relaxation was produced by atropine (10^{-6}g/ml). B; ADP (10^{-5}g/ml) was added after atropine. C; ADP ($2\times 1\ 10^{-5}$g/m) and D; ADP (3×10^{-5}g/ml) after atropine. Potentiations in the initial relaxation and the rebound-contraction were seen in B, C and D. The duration of single pulse given at the marks was 0.1 msec. Calibration, 10 sec and 0.5g.

ion. The magnitude of the relaxation was increased with the concentrations of the external appleid ATP (10^{-5}–3×10^{-5}g/ml). The rebound-contractions were also potentiated with the external ATP concentrations. Similar results were obtained by ADP (10^{-5}–3×10^{-5}g/ml) after atropine (10^{-6}g/ml). Fig. 7 shows the inhibitory effect of ADP on the contractile response to single transmural stimulation.

DISCUSSION

Three types of motor nerve fiber to the intestinal smooth muscle have been generally accepted. However the quantitvie distribution of these intrinsic nerves in various parts of the gut is unknown. The segment of the guinea-pig ileum used in the present experiment may include these different types of nerves. When single transmural stimulation was applied,

the primary contraction was produced in some preparations. The primary contractions are mediated by the cholinergic excitatory transmission because they are abolished by atropine treatment but not d-tubocurarine and are also abolished by hyoscine which is known to have a blocking effect on the excitatory junction potential[3),4),6),7)].

In some preparations, the relaxation type of the response was also observed by single transmural stimulation. The production of the different responses by equal transmural stimulation is considered as follows. Three different functional types of neurone are present in the intrinsic nerves of the gut. The quantitive distribution of these intrinsic nerves in the gut must be considered. When the trnasmural stimulation was given, the change in the membrane potential of each smooth muscle is determined by the nerves which are active at the time. In fact, Furness[2)] reported the biphasic change in the membrane potential of the smooth muscle of the guinea-pig colon when the intrinsic nerve fibers were stimulated.

When a single transmural stimulation is given, if the number of the active intrinsic excitatory nerves is dominant in the segment, a contraction may be recorede in the whole segment and if the number of the active intrinsic inhibitory nerve is dominant, relaxation is recorded. Of course, this is related not only to the number of the active nerves but also to the quantity of the transmitter substances and the contribution to the change in the membrane potential of each smooth muscle. That is, the region which produces the primary contraction is the excitatory dominant type and the other is the inhibitory dominant type. Treatment of the ileum with physostigmine led to decrease in the initial relaxation. This result suggests that the modification by physostigmine is made on the inhibitory dominant type. Munro[19)] reported that the guinea-pig terminal ileum normally produced a primary contraction. In the sense of dominant type, the part of the terminal ileum may be an excitatory dominant type.

An alternative view is the following. The mechanical response to single transmural stimulation was dependent on the tone of the preparation[2)]. The tone of the preparation may depend on the activity of the intrinsic nerves which are considered to be the neurones in Auerbach's plexus. It has been reported that the neurones in Auerbach's plexus showed single or burst activity without an external stimulation[13),14)]. The excitatory or inhibitory function of these neurones has estimated from the effects of autonomic drugs on them[14),15)]. Therefore the tone of the segment may depend on the activity of both functional types of neurones. Under the condition of high tone, the excitatory neurones may be more dominant and at low tone the inhibitory neurones are dominant. In the sense of tone,

the high tone preparation is an excitatory dominant type and the low tone preparation is an inhibitory dominant type. When a pulse is given to the high tone preparation, the pulse is more effective to the inhibitory neurones because the excitatory neurones are already active but many inhibitory neurones are in non-excited state. Thus the initial relaxation is produced in the high tone preparation. In contrast, when a pulse is given to a low tone preparation, the primary contraction may be produced. In fact, it has been reported that the mechanical responses, relaxation or contraction, to single, equal stimulation in one preparation are depend on its tone[2]. The results obtained from the guinea-pig colon[2] agree with the above consideration. It had also reported the guinea-pig ileum and colon were belonged to the excitatory dominant type[12].

The initial relaxation is mediated by the hyperpolarization of the smooth muscle cell membrane by transmitter substances from the adrenergic and/or non-adrenergic inhibitory nerves. In the previous papers[2)-7)], it is shown that the transmural stimulation to the intrinsic inhibitory nerves produced the hyperpolarization of the smooth muscle cell membrane. It is also reported that hyperpolarization is related with the rebound excitation[2),4)]. Since the initial relaxation was increased with the strength of single transmural stimulation in hyoscine, the increase in the strength may increase the release of transmitter substance and the number of excited intrinsic inhibitory nerves.

As shown in the results, the initial relaxation was partly inhibited by phenoxybenzamine, so the relaxation is partly due to the excitation of the adrenergic inhibitory nerves.

The results of the effects of ATP and ADP indicate that the relaxation is partly due to the non-adrenergic inhibitory nerves. It had been reported that the inhibitory junction potential in the intestinal smooth muscle was unaffected by guanethidine[5),6),7)] and that the relaxation in response to stimulation was not blocked by guanethidine[16)]. In the present experiment, guanethidine had no effect on the initial relaxation in normal solution and after atropine. This result also suggests that the initial relaxations are partly caused by the stimulation of non-adrenergic inhibitory nerves.

Rebound excitaion or after-contractions have been previously reported in the intestinal smooth muscle[1)-4),17),18)]. In the present experiment, the contractions which may correspond to the rebound excitation or after-contraction were obtained in normal state, after atropine or hyoscine. The contraction was observed after the repetitive stimulation in atropine or hyoscine. Moreover, it could be generated by single transmural stimulation to inhibitory nerves. In the present study, the contraction was termed

"rebound-contraction" because it was produced by single transmural stimulation. In the electrophysiological study on the rebound excitation in the intestinal smooth muscle cells, it reported that the inhibitory junction potentials were followed by either a single or burst action potentials[5),17)]. Action potentials in the smooth muscle cell membrane at the rebound excitation might be related to the rebound-contraction. The delay between the single transmural stimulation and the start of the rebound-contraction was longer than that in the primary contraction. The rebound-contractions were produced in atropine or hyoscine. From above considerations, the rebound-contraction is not mediated the nervous factors and it may depend on the recovery of the hyperpolarized membrane potential and the spontaneous generation of the action potentials.

The rebound-contraction is abolished by tetrodotoxin. This may due to block the activity of the intrinsic inhibitory nerves by tetrodotoxin. The block of the intrinsic inhibitory nerves may produced no hyperpolarization of the smooth muscle cell membrane. The generation of the rebound-contraction might require the preceded hyperpolarization and the process of the rebound excitation may be intrinsic in the smooth muscle cell membrane itself. This conclusion coincides with that of Bennett[3),4)] and Furness[2)] obtained from the guinea-pig taenia coli and colon but not agreed with that of other authors[18),20)].

It is concluded from the present experiment that three types of intrinsic nerves exist in the guinea-pig ileum and a non-cholinergic excitatory innervation of the guinea-pig ileum is unlikely and that rebound-contraction is not nerve-mediated phenomenon.

SUMMARY

1. A study was made of the mechanical responses of the isolated ileum of the guinea-pig to single transmural stimulation of the intrinsic nerves and to drugs.

2. Excitatory dominant and inhibitory dominant types of responses were observed. Rebound-contraction produced by single transmural stimulation was also demonstrated.

3. Atropine or hyoscine abolished the primary contraction and produced the relaxation type response. The initial relaxation was dependent on the tension level at which the single transmural stimulation was given.

4. TTX but not hexamethonium inhibited the primary contraction, the initial relaxation and the rebound-contraction by blocking the activity of the intrinsic nerves.

5. Phenoxybenzamine partly inhibited the initial relaxation and guanethidine had no effect on it. ATP and ADP potentiated the initial relaxation suggesting non-adrenergic inhibitory neurones in the guinea-pig ileum. The result supports that the possibility that ATP or a related compound is a transmitter substance released by non-adrenergic inhibitory nerves.

6. It is concluded that three types of intrinsic nerves are included in the wall of the guinea-pig ileum. Rebound-contraction appears to be a non-nerve-mediated phenomenon. The process of the rebound-contraction may be due to intrinsic mechanism in smooth muscle cells due to the preceding hyperpolarization.

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